

Transfection

Key words: Transient transfection, Stable transfection, transfection methods, vector, plasmid, origin of replication, reporter gene/ protein, cloning site, promoter and enhancer, signal peptide, polyadenylation signal.

- **Transfection**-Transfer of non-viral genetic material into eukaryotic cells.
- **Infection/ Transduction**- Transfer of viral genetic material into cells.
- **Transformation**- Transfer of genetic material into bacterial and plant cells.

Transfection

- The goal of transfection is to express a particular gene in the host cell.
- Used to Study : Gene expression regulations, Protein function, Gene silencing, Gene therapy and more.

Transient Transfection

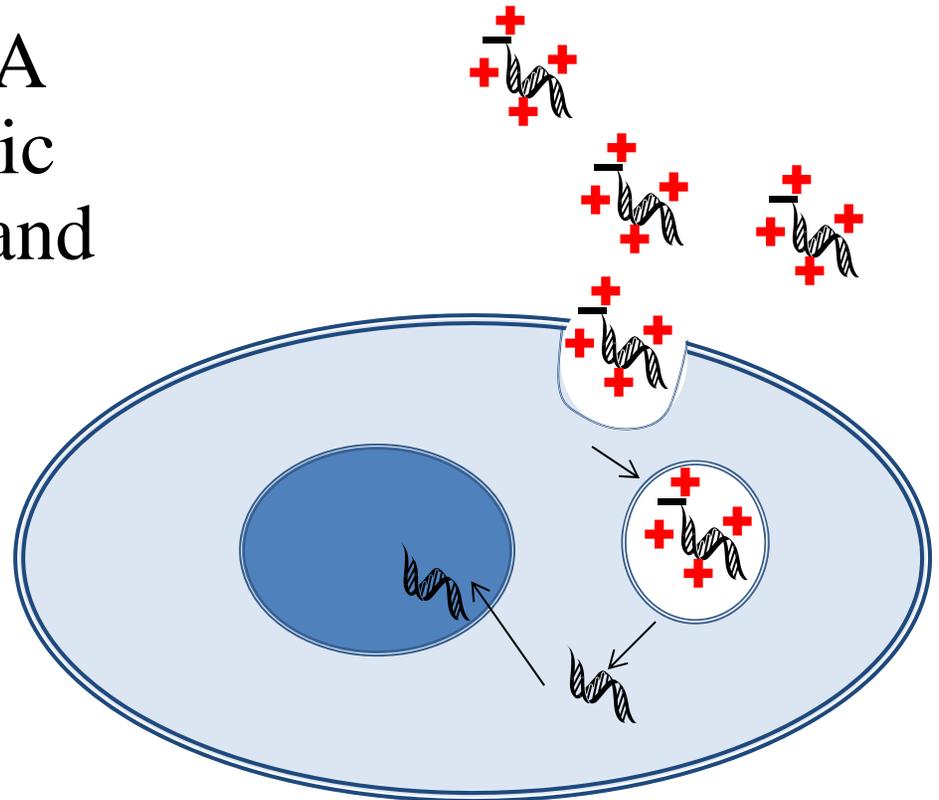
- When the introduced DNA molecule is not inserted into the host genome.
- The DNA is eventually lost during mitosis.
- Transiently transfected cells are assayed 48-72 hours after transfection.

Stable Transfection

- Occasionally the introduced DNA is inserted into the host genome. The DNA becomes part of the host genome and is passed to the next generations.
- The cells that are stably transfected need to be selected for and separated from the transient or non-transfected cells. The stably transfected cells are normally selected for about 48 hours post transfection.

Transfection Methods

- Negatively charged DNA interacts with the cationic molecules or polymers and is taken into the cell by endocytosis.
- Examples:
Calcium Phosphate
cationic liposomes



Other Transfection Methods:

Nanoparticles

- Nanoparticles are coated with DNA and directly shot into the nucleus.

Microinjection

- Direct injection into individual cells by a very thin needle.

Electroporation

- The electric potential across the membrane drives DNA across the membrane through the pores.
- Charged ions and DNA flow through the pores, then the cell membrane discharges and the pores close and the DNA molecules are inside the cell.

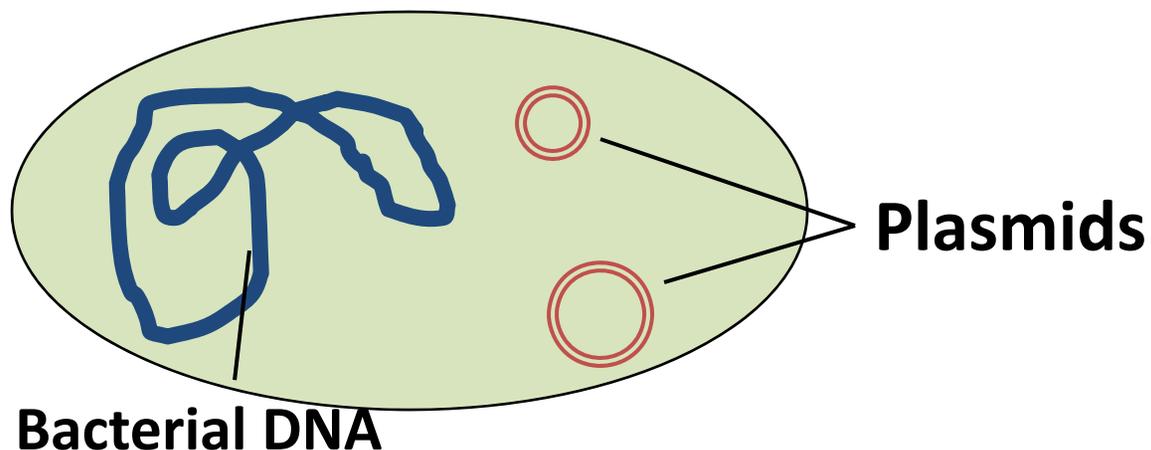
Vectors

- Carrier DNA sequences that are able to enter the host cells
- Able to replicate independently in the host cell
- Able to express genes in the host cell
- Plasmids, artificial chromosomes and viruses are commonly used as vectors.
- Non-viral vectors rely on cell division for transfer of DNA into the nucleus.

Plasmids:

Extra-chromosomal DNA molecules found in bacteria.

- Double stranded DNA
- Circular
- Carry genes with variety of functions



Plasmids are genetically engineered to be used as vectors that can carry foreign DNA.

Plasmids that are commonly used for biotechnology have the following features:

- Small size (1-500 kb)
- Origin of replication- Can replicate in the host cell and make high copy numbers
- Selectable marker gene
- Cloning site

Origin of Replication

- A sequence of DNA where replication is initiated.
- Plasmids are often amplified in bacteria and then used for transfection in other cell types. Therefore they possess two different origins of replication suitable for both cell types.
- Some origins of replication allow more efficient replications and yield high copy number of plasmids.
- **High copy number** origins are preferred since more plasmids are replicated in shorter time.

Cloning site

Refers to multiple restriction enzyme sites on the plasmid's sequence where foreign DNA can be inserted.

Reporter Gene (Selectable marker)

- A reporter gene used to select for the transfected cells and separate them from non-transfected cells.
- Antibiotic resistant genes or genes coding for fluorescent proteins are commonly used as selectable markers.

In addition plasmids may be genetically engineered to contain specific DNA sequences such as:

- **Enhancer and promoter sequences**
- **Signal peptides**
- **Polyadenylation sequence**

Enhancer and promoter sequences

- Regulatory sequences where transcription factors and regulatory proteins bind and control expression of a specific gene.
- Enhancer and promoter sequences of different genes may be specific to an organism and may be active only in specific tissues.
- Plasmids are engineered with appropriate regulatory sequences that are inserted upstream of the gene of interest (5' to the RE sites) specific to an organism and may be specific to a tissue.

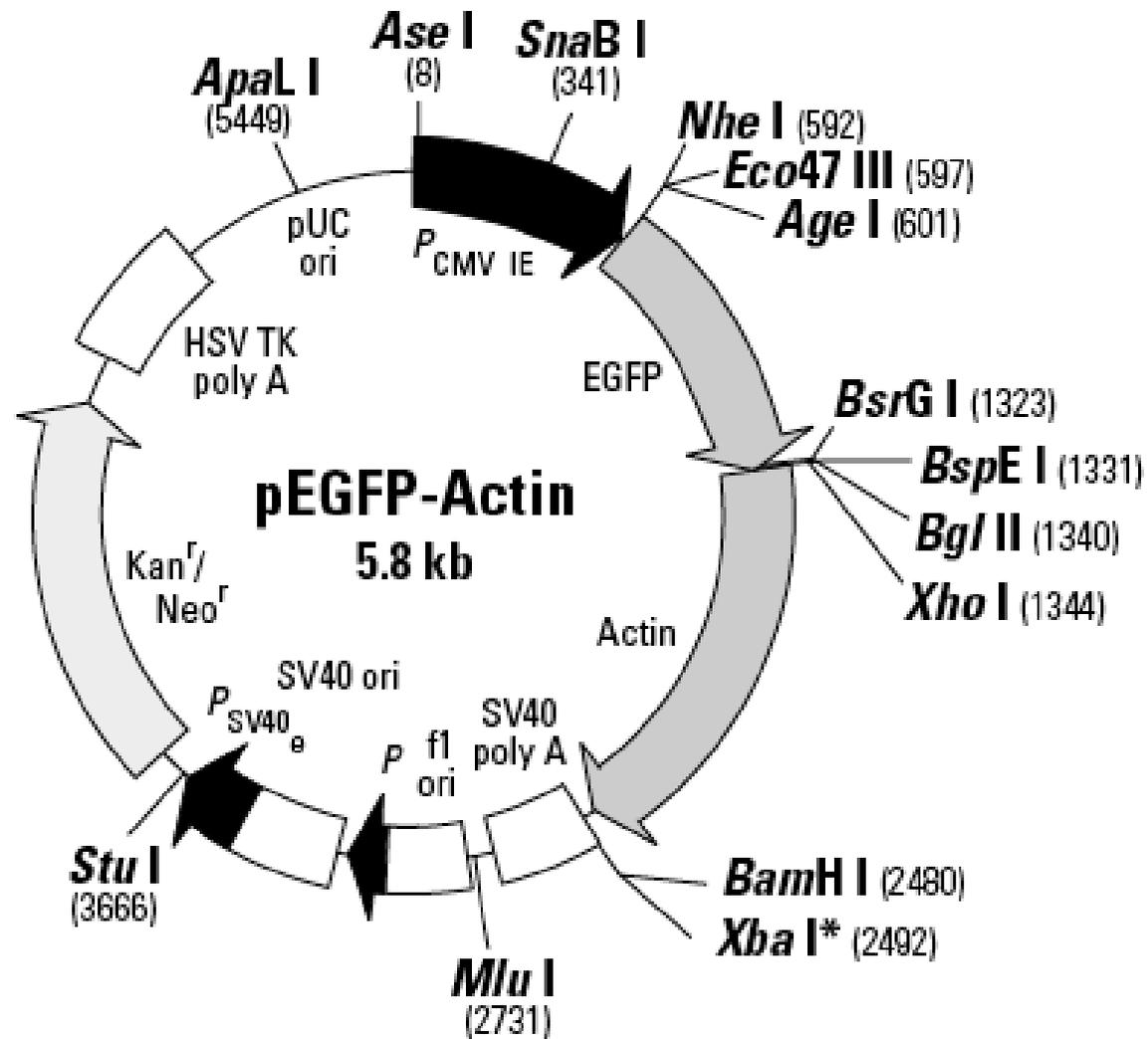
Signal peptides

- A short peptide sequence on every protein that used to interact with transport systems in the cell to direct the protein to the correct cellular location.
- Signal peptides are often cleaved off once the final destination has been reached.
- A plasmid may be engineered to carry a signal sequence to direct the protein product of the inserted gene to a specific location inside the cell or direct it to be secreted outside.

Polyadenylation sequence

- A specific sequence recognized by the polyadenylation machinery which adds a stretch of Adenines to the tail end (3') of the RNA molecule.
- RNA molecules that are polyadenylated are said to be matured (mRNA) and are more stable.
- Polyadenylation sequence may be inserted 3' to the cloning site to be used for polyadenylation of the RNA product.

pEGFP-Actin VECTOR



- **pUC Ori**- Origin of replication for propagation in bacteria.
- **SV40 Ori**- Origin of replication for replication in mammalian cells. Taken from the SV40 virus.
- **pCMV IE**- Cytomegalovirus promoter for gene expression in mammalian cells
- **pSV40**- SV40 promoter for gene expression in mammalian cells
- **P**- Bacterial promoter for gene expression in Ecoli bacteria
- **SV40 Poly A and HSV TK Poly A**- Polyadenylation and stabalization of the mRNA expressed in mammalian cells.
- **Neo/ Kan (r)**- Selectable genes that encode an enzyme to make both bacterial and mammalian cells resistant to gentamicin, neomycin and kanamycin. (These antibiotics kill cells by interfering with protein synthesis.)